THE BATTLE THAT SEEMED LOST

Nanoparticles against multiresistant *Pseudomonas aeruginosa*

Paula Bellés Sancho

INTRODUCTION AND BACKGROUND

Pseudomonas aeruginosa is a gramnegative bacteria which causes most of the nosocomial infections in humans. It is frequently associated with respiratory diseases, being the principal cause of mortality[1]. These infections are difficult to treat with antibiotics due to three main factors[2], shown in the following figure (**Figure 1**):

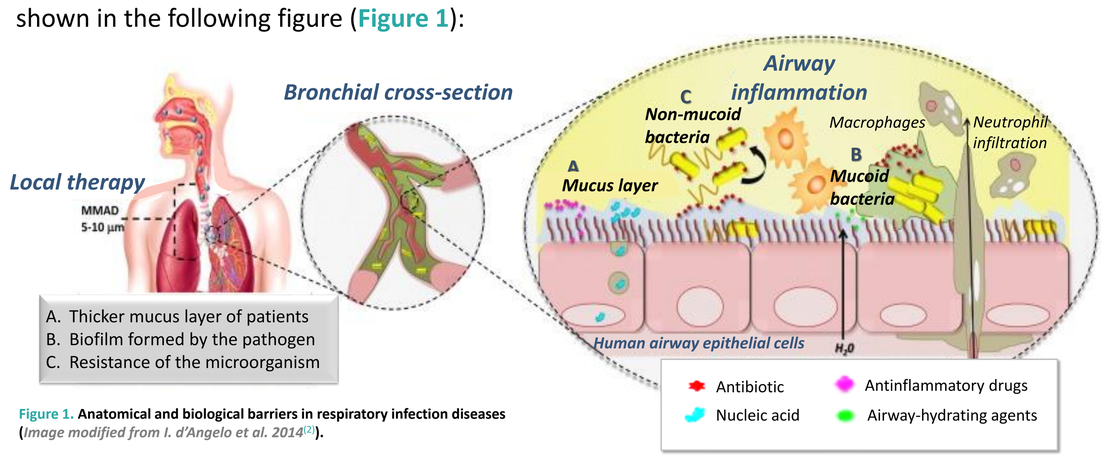


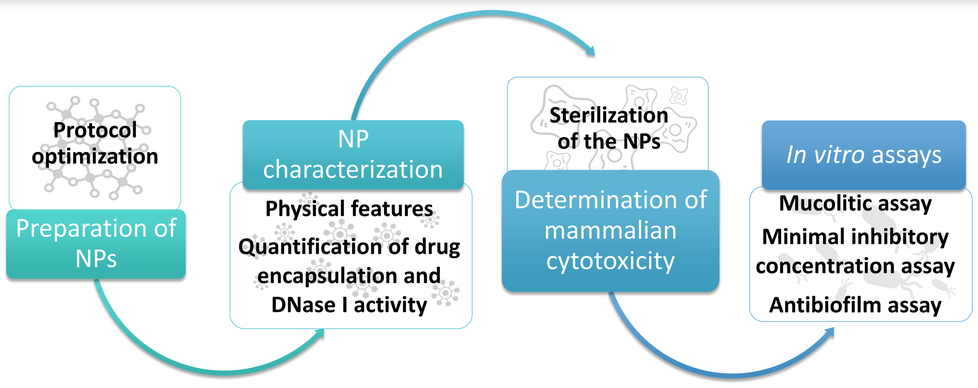
Figure 1. Anatomical and biological barriers in respiratory infection diseases (*Image modified from I. d'Angelo et al. 2014*[2]).

The combination of these factors and the low bioavailability that, in most cases, traditional drugs present, make the treatment ineffective. For this reason, new strategies based on nanocarriers carrying antimicrobial drugs, such as polymeric nanoparticles (NPs), have been studied to due to their potential to encapsulate and deliver the drug. These nanocarriers-based strategies are expected to increase bactericidal effectiveness, avoiding the side effects reported on traditional drugs[2].

OBJECTIVES

The aim of this project is:

- To develop a polymeric NPs based on poly(lactic-co-glycolic) acid (PLGA) and polyethylenimine (PEI). They will be modified by covalent grafting of deoxyribonuclease I (DNase I) and will encapsulate ceftazidime (CFZM).
- To assess the NP bactericidal potential against *P. aeruginosa* infection with three different *in vitro* assay (**Flowchart 1**).



Flowchart 1. Diagram of the specific objective. Each objective is considered as a work-package.

MATERIAL AND METHODS

Preparation of the PLGA/PEI–CFZM–DNase I NPs

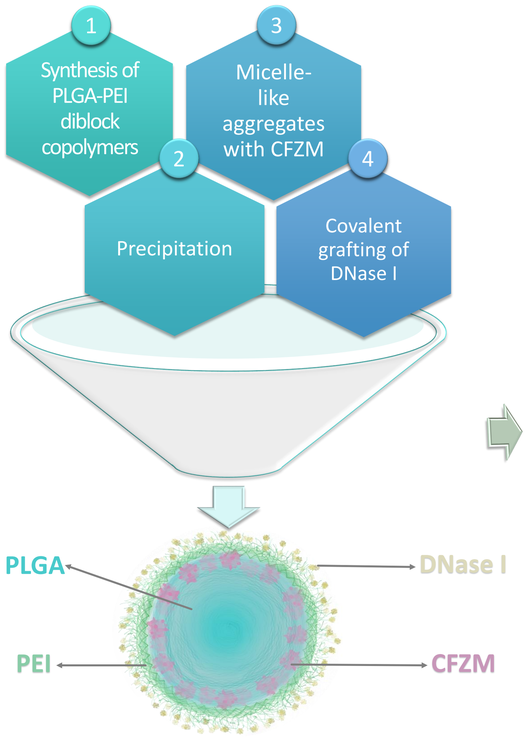


Figure 2. NPs preparation diagram based and modified from *Y. S. Nam et al. 2003*[3] (1, 2 and 3) and *A. Baelo et al. 2015*[4] (4). The NPs resulting is an amphiphilic NP based on PLGA-PEI polymer and modified with Dnase I and CFZM.

Characterization

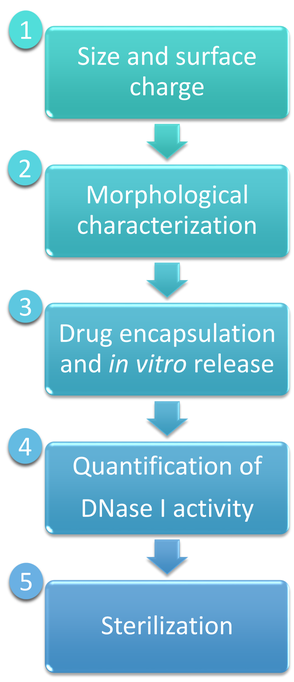


Figure 3. Flowchart on NPs characterization, baseded and modified from *A. Baelo et al. 2015*[4] (1, 2, 3 and 4). Step 5 is based on *M. A. Vetten et al. 2014*[5].

Mammalian cytotoxicity

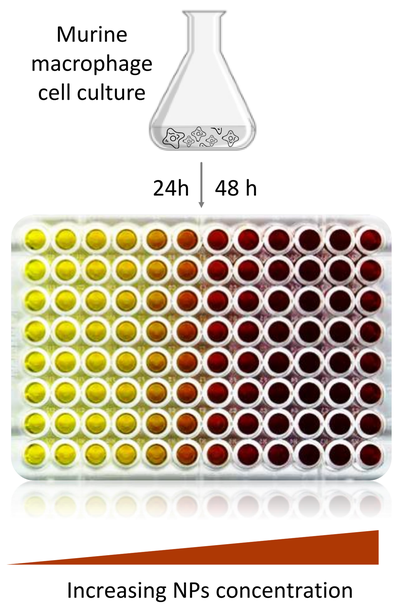


Figure 4. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) colorimetric assay after 24 h and 48 h of exposure to different NPsconcentrations, tested in murine macrophage cells. Based on *A. Baelo et al. 2015*[4].

In vitro assays

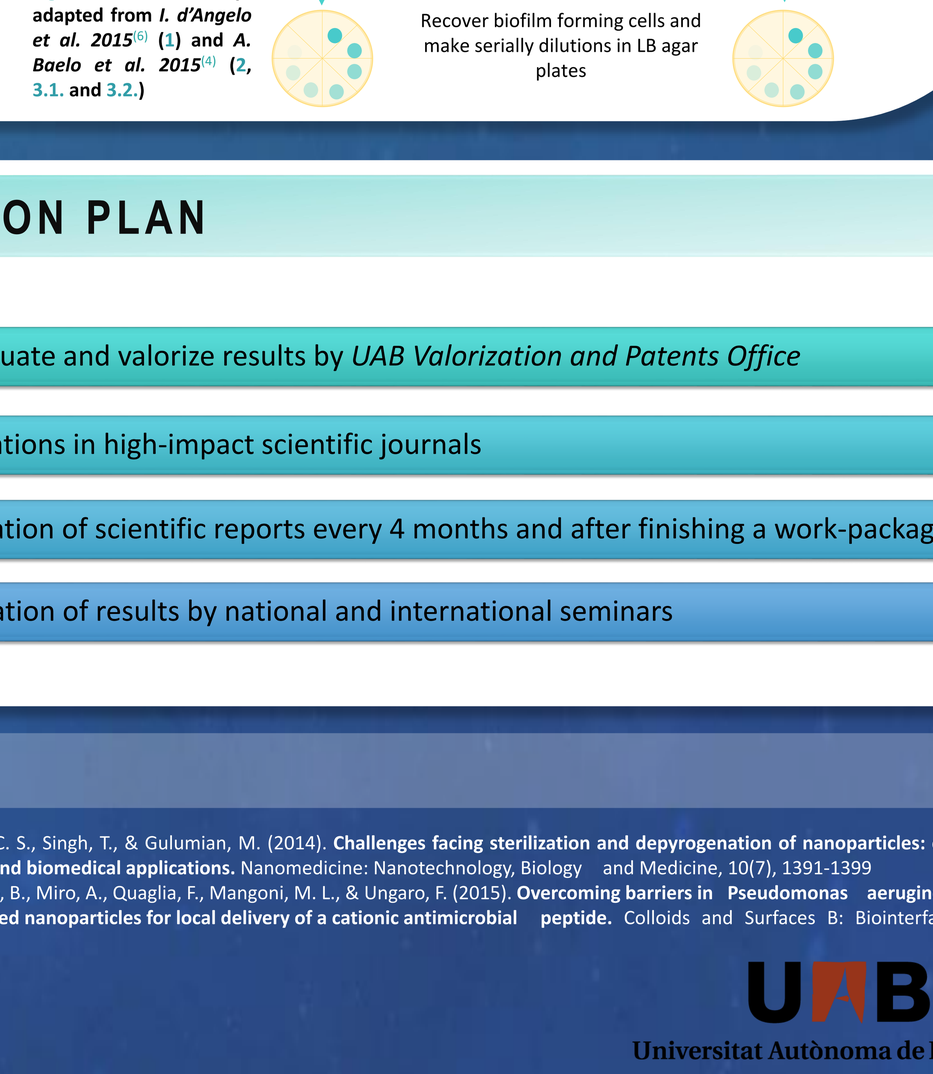


Figure 5. *In vitro* assays adapted from *I. d'Angelo et al. 2015*[6] (1) and *A. Baelo et al. 2015*[4] (2, 3.1. and 3.2.)

EXPECTED RESULTS

Results of *in vitro* assays are represented in **Figure 6**. Each expected result is related to a component of the NPs, based on previous studies[2, 4].

This project is designed for 3 years. Results are expected to be obtained in the last stage of the third year.

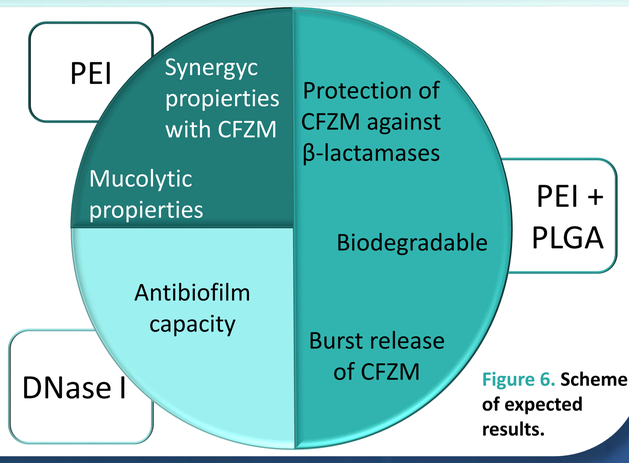


Figure 6. Scheme of expected results.

DIFUSION PLAN

- To evaluate and valorize results by *UAB Valorization and Patents Office*
- Publications in high-impact scientific journals
- Generation of scientific reports every 4 months and after finishing a work-package
- Divulgation of results by national and international seminars

BIBLIOGRAPHY

1. Lyczak, J. B., Cannon, C. L., & Pier, G. B. (2002). **Lung infections associated with cystic fibrosis.** Clinical microbiology reviews, 15(2), 194-222.
2. d'Angelo, I., Conte, C., La Rotonda, M. I., Miro, A., Quaglia, F., & Ungaro, F. (2014). **Improving the efficacy of inhaled drugs in cystic fibrosis: challenges and emerging drug delivery strategies.** Advanced drug delivery reviews, 75, 92-111.
3. Nam, Y. S., Kang, H. S., Park, J. Y., Park, T. G., Han, S. H., & Chang, I. S. (2003). **New micelle-like polymer aggregates made from PEI–PLGA diblock copolymers: micellar characteristics and cellular uptake.** Biomaterials, 24(12), 2053-2059
4. Baelo, A., Levato, R., Julián, E., Crespo, A., Astola, J., Gavaldà, J., ... & Torrents, E. (2015). **Disassembling bacterial extracellular matrix with DNase-coated nanoparticles to enhance antibiotic delivery in biofilm infections.** Journal of Controlled Release, 209, 150-158.
5. Vetten, M. A., Yah, C. S., Singh, T., & Gulumian, M. (2014). **Challenges facing sterilization and depyrogenation of nanoparticles: effects on structural stability and biomedical applications.** Nanomedicine: Nanotechnology, Biology and Medicine, 10(7), 1391-1399
6. d'Angelo, I., Casciaro, B., Miro, A., Quaglia, F., Mangoni, M. L., & Ungaro, F. (2015). **Overcoming barriers in Pseudomonas aeruginosa lung infections: Engineered nanoparticles for local delivery of a cationic antimicrobial peptide.** Colloids and Surfaces B: Biointerfaces, 135, 717-725.

Universitat Autònoma de Barcelona